

Nitrogen Partitioning and Mobilization Patterns in Bean Plants¹

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ABSTRACT

The assimilation and distribution of N in the vegetative and reproductive plant parts of edible grain legumes are important processes determining final seed and protein yields. Limited information is available describing these processes for beans (*Phaseolus vulgaris* L.). The objective of this study was to measure the N partitioning and mobilization patterns in this important grain legume. Two dry bean cultivars, '3512' and '3591', were labeled with either $K^{15}NO_3$ or $^{15}N_2$ at the late vegetative (V5), early pod development (R2-R3), and seed filling (R6) developmental stages in the greenhouse. Initial N partitioning was evaluated on plants harvested 48 h after the start of labeling, while the N mobilization patterns were evaluated on the $^{15}NO_3$ -labeled plants harvested at subsequent developmental stages and at physiological maturity (R9). Both cultivars had similar dry weight and N distribution patterns throughout development, but 3512 had a larger final seed yield while the final seed N concentrations were greater for 3591. These differences developed from R6 to R9 where the dry weight increase in 3591 had a N concentration of 28.1 g kg^{-1} compared with 9.9 g kg^{-1} for 3512. The relative proportion of ^{15}N in each plant part was dependent upon the growth stage and ^{15}N source, and was independent of cultivar. Greater proportions were found in the mature leaves and roots of plants labeled with $^{15}NO_3$, and in the seed and nodules in those plants labeled with $^{15}N_2$ at all growth stages. Pods and seeds were major sinks from both ^{15}N sources when applied at R2-R3 and R6, respectively. The seed at R9 contained 64, 73, and 84% of the labeled-N applied at the V5, R2-R3, and R6 growth stages, respectively. The seed contained an average of 68% of the total plant N and 53% of the total plant dry weight at R9. These data indicate that the photosynthetic and N_2 -fixation activities during seed filling can have a significant influence on the final seed N concentrations and yield.

Additional index words: Dry matter, Plant parts, *Phaseolus vulgaris* L., N uptake, Plant development, ^{15}N sources.

THE assimilation and distribution of N in the vegetative and the reproductive parts of edible grain legumes are important processes in determining seed yields. Significant cultivar differences in the relationships between vegetative N, seed yields, and seed protein concentrations are reported for beans

(*Phaseolus vulgaris* L.) (20) and soybean [*Glycine max* (L.) Merr.] (6). However, efforts to increase both seed yields and protein concentration have generally not been successful because of their inverse relationship (8, 20). An increase in the total seasonal N_2 -fixation or total N uptake may not always increase seed protein concentrations because of this relationship. This paradox has stimulated numerous studies on the transport and utilization of C and N in grain legumes (2, 10, 11, 12, 14).

Limited information is available for beans that describes the initial partitioning of symbiotic and combined N sources and their mobilization patterns during seed development. Identification of the differences between cultivars with similar seed yields and different seed N concentrations should provide a better understanding of the sequence of events and processes giving rise to higher seed protein (N) concentrations and aid in future selections. The purpose of this study was to examine the initial partitioning and the subsequent mobilization patterns of dinitrogen and combined N in beans during growth and development.

METHODS AND MATERIALS

Two cultivars, '3591' and '3512', selected from 20 advanced generation lines were used in this study. These pinto seed types of the same maturity class had similar yields but different seed N concentrations when grown under field conditions (20).

Sized seed between 0.36 and 0.46 g seed⁻¹ were germinated in perlite. Individual plants were then selected for uniformity of top growth and transplanted to 3.6 L plastic pots containing perlite when the primary leaves were fully expanded at about 10 days after planting. The plant roots were inoculated by dipping them in a water-rhizobial inoculum (Nitragin Co., Milwaukee, WI³) slurry before transplanting. Each pot had a hole in the bottom for drainage and was isolated from other pots on the greenhouse bench by placement on individual aluminum-foil pans.

After transplanting, 5 mg N as NH_4NO_3 was applied to each plant-pot in 0.2 L nutrient solution (15). Thereafter individual pots were watered daily to a predetermined wet weight alternating with distilled water and a nutrient solution containing 5 mg N kg⁻¹. The volume of nutrient solution containing N was recorded and a total of 21.6 mg N (including labeled-N) was applied to all plants reaching physiological maturity. This rate and amount of combined

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³ Company and commercial names are shown for the benefit of the reader and do not imply endorsement or preferential treatment of the product listed.

N did not appear to visibly inhibit nodulation. The individual pots were also completely re-randomized on the greenhouse bench twice each week. Daylight was supplemented with high pressure Na-lights for 12 h. Maximum greenhouse air temperatures averaged 32°C from 1 April to 25 June 1980, with short intervals up to 38°C, while minimum air temperatures reached about 18°C during the study.

The ^{15}N was applied at three plant developmental growth stages, V5, R2-R3, and R6, corresponding to late vegetative, early pod development, and seed filling, respectively (9). Individual plants were given 4.9 mg ^{15}N as K^{15}NO_3 ($^{15}\text{NO}_3\text{-N} = 83.39\%$) in 0.01 L distilled water followed by an application of 0.2 L distilled water to move the labeled-N into the root system. All plants not receiving the ^{15}N at each developmental stage were given 2 mg N as NH_4NO_3 in 0.2 L water. After 48 h the pots of all plants were leached with 2 L of distilled water to remove any residual $^{15}\text{NO}_3\text{-N}$ and other soluble salts, and the leachate discarded. Average plant recovery of the added $^{15}\text{NO}_3\text{-N}$ was 80%.

Three plants of each cultivar were also treated with $^{15}\text{N}_2$ at each developmental stage to determine the initial partitioning of symbiotically fixed N. Each pot was placed into a Saran³ bag, the bag sealed along the stem with plumber's putty (Duct seal³), partially evacuated and then back-filled with approximately 1 L of 98 atom % excess $^{15}\text{N}_2$ gas. This procedure gave an internal enrichment during the incubation of about 39 atom % excess $^{15}\text{N}_2$. These plants were incubated in the bags for 24 h, and then harvested 24 h after the end of the incubation period.

Three labeled plants from each cultivar were harvested 48 h after the start of labeling, at subsequent growth stages, and at physiological maturity (R9). Plants were separated into roots, nodules, stems, pod walls, seeds, and young, mature, primary, and old leaves. Mature leaves were defined as leaves that were fully expanded, while old leaves showed visible signs of senescence. Old leaves and petioles which abscised from individual plants were saved for dry weight and N analysis. All parts were dried at 60°C, weighed, ground to pass a 40-mesh sieve, and analyzed for total N by a semimicro Kjeldahl procedure modified to include $\text{NO}_3\text{-N}$ (1). The plant N was also analyzed for atom percent ^{15}N with an AEI MS-20 isotope-ratio mass spectrometer, using conversion techniques (13). The atom % excess ^{15}N in each plant part was calculated from the equation $[N_T(a-b)/(c-b)]$, where N_T is the N content of the plant part, a and b are the atom percent excess ^{15}N of the treated and non-treated plant parts, and c is the atom percent excess ^{15}N of the added N material (5). Statistical analyses were carried out using a factorial, completely randomized design with three replications for each sampling. Duncan's Multiple Range Test was used to make comparisons among treatment means.

RESULTS

The average dry matter distribution in the plant parts were generally similar for both cultivars at each plant developmental stage (Fig. 1A). A significant difference in total plant dry matter between cultivars only occurred at R9 because of greater seed yields for 3512 compared with 3591 (Table 1). The dry weights of the pod walls for 3512 were also greater than those for 3591 at the R6 developmental stage. The average percentage distributions of the dry weight of the plant parts at all developmental stages were similar for both cultivars, although 3512 had about 54% of its total dry weight at R9 in the seed compared to 51% for 3591.

The total N content of the plant parts was similar for both cultivars at all plant developmental stages (Fig. 1B). The seed of 3591 contained an average of 68% of the total plant N compared with 70% for 3512 at R9 (calculated from data in Table 1). The N concentrations of the old leaves and seed were only significantly different between the two cultivars at R9. The seed and the old leaves of 3591 contained 34.5 and 15.4 g N kg⁻¹ and for 3512 they contained 30.1 and 12.6 g N kg⁻¹, respectively. There was no significant cultivar by plant part interaction for N content at any growth stage.

The average percentage distributions of the ^{15}N in the respective plant parts for the two cultivars 48 h after the K^{15}NO_3 and $^{15}\text{N}_2$ applications are shown in Table 2. There were no significant differences be-

Table 1. The distribution of dry weight and N content in the plant parts for two bean cultivars (3512 and 3591) at two plant developmental stages.

Culti- var	Plant part	Development stage				Differences between R9-R6	
		R6		R9		Dry wt	N
		Dry wt	N	Dry wt	N		
		g					
3512	Nodules	0.59	0.023	0.24	0.012	-0.35	-0.011
	Roots	0.92	0.021	0.84	0.021	-0.08	0.000
	Seed	1.07	0.038	4.58	0.138	3.51	0.100
	Pod walls	2.63	0.052	1.15	0.009	-1.48	-0.043
	Stems	0.66	0.007	0.43	0.003	-0.23	-0.009
	Old leaves	0.24	0.005	1.19	0.015	0.95	0.010
	Mature leaves	0.98	0.033	-	-	-0.98	-0.033
	Young leaves	0.23	0.008	-	-	-0.23	-0.008
	Total plant	7.32a	0.187a	8.43b	0.198a	1.11	0.011
3591	Nodules	0.43	0.018	0.24	0.011	-0.19	-0.007
	Roots	0.96	0.021	0.77	0.019	-0.19	-0.002
	Seed	1.27	0.044	3.68	0.127	2.41	0.083
	Pod walls	1.86	0.039	0.96	0.009	-0.90	-0.030
	Stems	0.59	0.007	0.34	0.002	-0.25	-0.005
	Old leaves	0.40	0.008	1.17	0.018	0.77	0.010
	Mature leaves	0.74	0.023	-	-	-0.74	-0.023
	Young leaves	0.27	0.008	-	-	-0.27	-0.008
	Total plant	6.52a	0.168a	7.16a	0.186a	0.64	0.018
LSD _{0.05} †		0.67	NS	0.62	NS	-	-

† For comparison of plant parts between cultivars within a developmental stage. Total plant means for each cultivar within a developmental stage followed by different letters are significantly different at the 95% probability level.

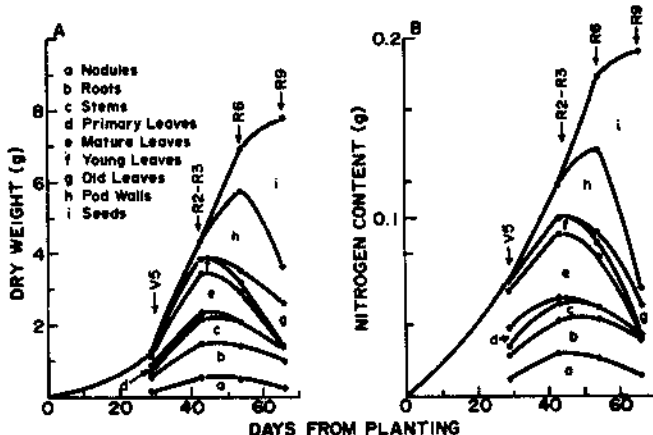


Fig. 1. The distribution patterns of dry weight (A) and N content (B) in the plant parts averaged across cultivars (V5, R2-R3, R6, and R9 = late vegetative, early pod development, seed filling and physiological maturity plant developmental stages, respectively).

tween cultivars in percentage distribution among plant parts within either ^{15}N source. The proportion of ^{15}N in each plant part was dependent upon the developmental stage and the ^{15}N source. The plant parts containing the highest proportion of ^{15}N from the $^{15}\text{NO}_3\text{-N}$ source were mature leaves, roots, pod walls, and seeds, while the pod walls and seeds were the highest for the plants given the $^{15}\text{N}_2$. Consistent differences between ^{15}N sources occurred for mature leaves, roots, and nodules. Similar distribution differences among plant parts for N_2 and $\text{NO}_3\text{-N}$ sources were also reported for soybeans (7,21).

The mobilization of the ^{15}N from the $^{15}\text{NO}_3\text{-N}$ source with plant developmental stage is shown in Fig. 2. The averages for the two cultivars are shown since the cultivar by plant part interaction was not significant. These data show that about 64, 73, and 84% of the ^{15}N was in the seed at R9 when it was applied at the V5, R2-R3, and R6 developmental stages, respectively. This indicates that proportionally more N remained in the vegetative portions of the plant when the ^{15}N was applied to the plant at successively earlier developmental stages. Significantly higher ^{15}N enrichments were also found at R9 in the old leaves, pod walls, and roots when the $^{15}\text{NO}_3\text{-N}$ was applied at V5 or R2-R3 compared with the R6 application (data not shown).

The initial experimental plans were to apply equivalent N amounts to all plants at each developmental stage, however, an experimental error caused 2.9 mg

more N to be applied to the plants receiving the labeled-N at each developmental stage. The accumulated total amount of combined N applied, labeled plus non-labeled, was the same for all the plants harvested at the R6 and R9 developmental stages. Since there was no significant cultivar by growth stage interaction, specific effects were averaged across cultivars. Data at the R9 harvest showed that the plants treated at V5 and R2-R3 compared to those treated at R6 had significantly larger N contents and dry weights for both seed and total plant production at R9 (Table 3). The proportion of the total plant N found in the seed was also significantly greater for the plants treated at V5 and R2-R3 compared with the R6 treated plants. The differences in dry weight and N content were reflected by more nodes and pods for the V5 and R2-R3 treated plants. The higher N application had no significant effects on the N concentrations of the plant parts at any harvest.

DISCUSSION AND SUMMARY

The two cultivars, 3512 and 3591, were selected for their record of producing similar seed yields with different seed N concentrations under field conditions (20). The results of this greenhouse study again demonstrated the inverse relationship between seed yields and N concentrations (protein concentration). The inverse relationship between seed yields and N concentrations developed between R6 and R9. Comparison of the changes in the dry weights and N contents for the total plant and seed between R6 and R9 showed that 3512 mobilized a larger amount of dry weight and N from its vegetative tissue to the seed than did 3591 during seed filling. Sink strength if measured by pods per plant or seeds per pod was

Table 2. The ^{15}N distribution 48 h after the start of labeling expressed as a percentage of the total ^{15}N in the plant at three developmental stages for two ^{15}N sources. Averaged across cultivars.†

Plant part	Growth stage					
	V5		R2-R3		R6	
	¹⁵ N source					
	NO ₃	N ₂	NO ₃	N ₂	NO ₃	N ₂
	%					
Young leaves	11.50a	12.22a	10.36a	12.44a	7.70a	3.72a
Mature leaves	40.00b	6.13a	27.25b	14.37a	15.78b	7.24a
Primary leaves	7.82a	2.64a	1.34a	0.62a	—	—
Old leaves	—	—	—	—	2.43a	0.10a
Stems	10.36a	28.05b	8.57a	6.70a	6.22a	3.70a
Pod walls	—	—	28.20a	36.66b	20.33b	13.40a
Seeds	—	—	—	—	31.42a	53.28b
Roots	28.04b	16.02a	20.38b	5.86a	13.84b	4.52a
Nodules	2.28a	34.94b	3.89a	23.35a	2.28a	14.04b

† Percentages within a developmental stage and plant part followed by a different letter are significantly different at the 95% probability level.

Table 3. Effect of 2.9 mg N extra per plant at the respective developmental stages on final plant characteristics (R9). Averaged across cultivars.†

Treatment growth stage	Plant characteristic							
	Nitrogen content				Dry weight			
	Total		Seed/Total		Total		Seed/Total	
	mg	mg	mg	mg	g	g	g	g
V5	227a	158a	0.698a	9.2a	4.9a	0.533a	6.2a	5.1a
R2-R3	216a	150a	0.694a	8.6a	4.6a	0.535a	5.7ab	5.7a
R6	135b	89b	0.659b	5.6b	2.9b	0.518b	5.0b	4.0b

† Values followed by a different letter are significantly different at the 95% probability level.

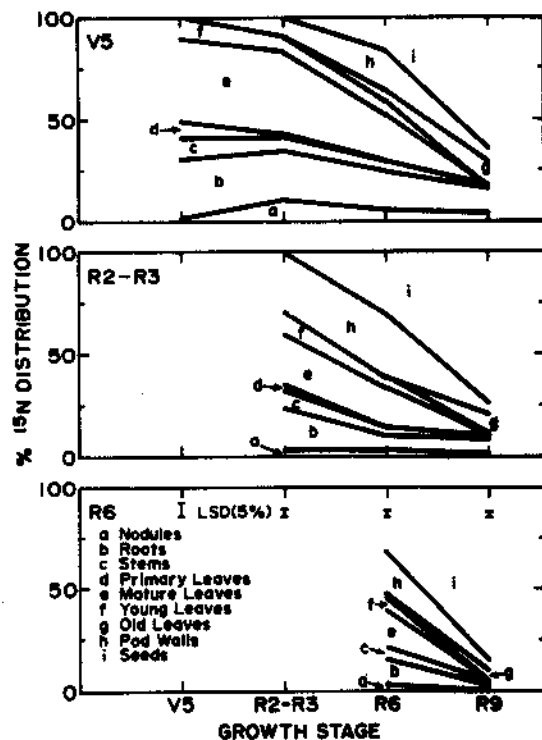


Fig. 2. The relative distribution of ^{15}N in the respective plant parts when labeled with $^{15}\text{NO}_3\text{-N}$ at growth stages V5, R2-R3, or R6.

similar for both cultivars (data not shown). The ^{15}N data for R6 and R9 also showed that 3512 mobilized approximately 15% more of the ^{15}N found in the vegetative tissue at R6 into the seed at R9 than did 3591.

The N concentration of the total plant for 3591 was significantly larger than that for 3512 at R9, but not significantly different at R6 (data not shown, but can be calculated from Table 1). This was largely caused by the N concentration in the total plant dry matter increase between R6 and R9 for 3512 (9.9 g N kg $^{-1}$) being appreciably less than that for 3591 (28.1 g N kg $^{-1}$). The total plant dry matter increase between R6 and R9 for 3512 was 1.7-fold larger than that for 3591, while the total plant N content increase for 3591 was 1.6-fold larger than that for 3512 during the same time interval (Table 1). If it is assumed that the dry matter and N content loss from the plant parts between R6 and R9 was mobilized to the seed, then the N concentration of this mobilized dry matter would be approximately 37 g N kg $^{-1}$ for both cultivars. These differences suggest that part of the lower seed N concentration for 3512 was caused by the diluting effect of the dry matter produced between R6 and R9.

An additional factor might be from different N_2 -fixation activities since no additional combined-N was applied after the R6 sampling. Preliminary data indicate that the nitrogenase activities (nmol C_2H_4 plant $^{-1}$ s $^{-1}$) for 3591 were 1.6 and 5.0-fold greater than those for 3512 at the R6 and R8 developmental stages, respectively (unpublished data, D. T. Westermann). These differences may be related to a significant cultivar by Rhizobial strain interaction, different photosynthetic activities, or photosynthate partitioning.

More N was contained after 48 h in the developing pods and seeds from N_2 -fixation than from $\text{NO}_3\text{-N}$, while the mature leaves and roots contained more N from $\text{NO}_3\text{-N}$ after 48 h. The forms of N being transported and how they are utilized by the plant are thought to cause some of these differences (21); e.g., the mature leaves contained a higher proportion of N from $\text{NO}_3\text{-N}$ because they are the main site of $\text{NO}_3\text{-N}$ reduction in this legume (3). A longer chase period (> 48 h) would probably decrease these differences since a significant portion of the $^{15}\text{NO}_3\text{-N}$ absorbed at all developmental stages was in the seed at R9 (Fig. 2). These data emphasize the importance of the N_2 -fixation activity during the pod development and seed filling processes on seed protein concentrations under field conditions, since more combined-N would normally be available during the vegetative developmental stages of this legume (18).

The proportion of the total N and dry matter found in the seed at R9 in this study compared favorably with the results of other studies for beans and soybeans grown under field conditions (4, 17, 18). Maximum plant sizes and seed yields per plant were smaller than normally found under field conditions which were probably a reflection of higher average night temperatures compared to normal field conditions (16).

The increase in final seed yield and plant sizes at

R9 from the 2.9 mg more N per plant at V5 and R2-R3 indicated that N was probably limiting early growth although all plants were well nodulated and there were no visible signs for N-deficiency. Nitrogen was shown to be more limiting than C in the early vegetative growth of soybeans (19). These data also support the seed yield responses obtained from small amounts of preplant fertilizer N under field conditions (18). The yield response from the earlier N applications resulted from an increase in plant size with a larger number of pods per plant. The proportion of N mobilized to the seed also decreased as the N was applied earlier in the growth cycle. This suggests that higher seed protein concentrations might be obtained from a combined N application during seed fill (3) or by selecting cultivars that are able to maintain a higher N_2 -fixation activity during seed filling.

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